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Lack of Association Between Tumor Necrosis and hsp-27 Expression in Primary Breast Cancer

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Overexpression of heat shock protein 27 (hsp-27) is associated with reduced disease-free survival in early stage breast cancer. Histopathologic evidence of confluent necrosis within primary infiltrating ductal carcinoma (IDC) is similarly an indication of poor prognosis. We postulated that IDC evidencing confluent tumor necrosis (TN) might overexpress this protein, which would help explain why hsp-27 is associated with higher-risk cancers. To test this hypothesis, presence of TN (as opposed to individual cell apoptosis) and of hsp-27 expression by immunohistochemistry were evaluated independently in 48 specimens of IDC. Nineteen (40%) overexpressed hsp-27 and 10 (21%) displayed necrosis. IDCs with areas of TN are less likely to overexpress hsp-27, suggesting a lack of association between these histoprognostic variables. This negative correlation, however, supports hsp-27 as an independent predictor of high-risk disease.

KEY WORDS: **heat shock protein 27, hsp-27, tumor necrosis, breast cancer**

INTRODUCTION

In multivariate analysis of variables including tumor size, estrogen receptor (ER) status, DNA ploidy status, and patient age, both heat shock protein 27 (hsp-27) overexpression and negative ER content were independent prognostic factors for shortened disease-free survival in early stage breast cancer [1]. Overexpression of hsp-27 was associated with factors suggesting a higher metastatic potential (e.g., cathepsin D), poor differentiation (e.g., DNA aneuploidy and high nuclear grade), and an increased probability of recurrence [2].

Tumor size and lymph node status are by consensus the most reliable predictors of outcome. However, tumor-specific factors may also have substantial bearing in determining risk of disease recurrence. ER content has prognostic value, but there is no substantial correlation between ER status and hsp-27 expression in infiltrating ductal carcinoma (IDC) [3]. Overexpression of the cell membrane oncoprotein HER-2/*neu* also has prognostic value in IDC [4]. However, no association between HER-2/*neu* overexpression and hsp-27 expression has been found in IDC or in ductal carcinoma in situ (DCIS) [5]. Histologic grade using Scarff-Bloom-Richardson criteria and lymph node status have similarly been found to have no correlation with hsp-27 expression [6].

We postulated that IDC evidencing confluent tumor necrosis (TN) might overexpress this protein, which would help explain why hsp-27 is associated with higher-risk cancers [7]. To test this hypothesis, the presence of TN (as opposed to individual cell apoptosis) and of hsp-27 expression by immunohistochemistry, were evaluated within the same IDC.

PATIENTS AND METHODS

Patients

Women with T 1–2, NØ-N1, MØ IDC consecutively treated by the authors between 1991 and 1994 with avail-

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able tissue samples were identified from the University of Wisconsin Hospital Tumor Registry. H&E sections and paraffin blocks of the primary tumor were retrieved and encoded for blinded evaluation. Sufficient invasive cancer remained for immunohistochemical analysis in 48 samples, which formed the database.

TN

Sections of the primary IDC were evaluated independently for TN using specific criteria of readily recognized confluent areas of tumor cell necrosis at ×40 magnification and excluding sites of scarring or apoptotic bodies [7].

Immunohistochemistry

Hsp-27 monoclonal antibody clone G3.1 (BioGenex Laboratories, Dublin, CA) was used. G3.1 is an IgG class mouse monoclonal antibody from ascites, diluted in phosphate-buffered saline (PBS), pH 7.6, with 1% bovine serum albumin (BSA), and preserved with 0.1% sodium azide [8]. StrAviGen Super Sensitive Detection kits, which use a biotin-streptavidin amplified detection system, alkaline phosphatase substrate (which does not require peroxide blocking), and chromogen kits were obtained commercially (BioGenex). Five micrometer sections were heat fixed on slides at 55°C for 2 hours, deparaffinized in xylene, then rehydrated through 100% and 95% alcohol to PBS, pH 7.6. Two drops of undiluted primary antibody or negative control (mouse) nonimmune serum were added to separate test and control specimens, and were incubated at room tempearture for 45 minutes. Positive control specimens were derived from archival breast cancer paraffin blocks of the same vintage, processed and preserved in the same manner as the test specimens, and previously tested for hsp-27 expression against commercial controls (BioGenex). After washing in PBS, two drops of link (biotinylated anti-mouse immunoglobulins) were added and incubated at room temperature for 20 minutes. After washing, two drops of label (concentrated alkaline phosphatase-conjugated streptavidin) were applied and incubated for 20 minutes, followed by a PBS wash, and then addition of two drops of New Fuchsin permanent substrate solution at room temperature for 40 minutes. Sections were washed in distilled water (DH_2O), counterstained in Mayer's hematoxylin for 60 seconds, ripened in tap water, rinsed in DH_2O, then air dried. Slide covers were affixed with permanent mounting media.

Immunostaining of hsp-27 in viable portions of the IDC was interpreted independently of investigation for TN, according to the method of Thor et al. [9]: nonreactive (<1% stained viable tumor cells), low expression (1–9% cells), or overexpression (10–95% reactive cells).

TABLE I. Hsp-27 Expression Vs. TN in Primary IDC of the Breast

	TN		
Hsp-27	Present (%)	Absent (%)	Totals (%)
Overexpression	3 (16)	16 (84)	19 (40)
Low or no expression	7 (24)	22 (76)	29 (60)
Totals (%)	10 (21)	38 (79)	48 (100)

Statistics

The strength of association between hsp-27 and necrosis was estimated with the odds ratio [10]. An odds ratio of greater than 1 indicates positive association (hsp-27 and necrosis both positive and negative), while an odds ratio of less than 1 indicates negative association (the presence of one condition is associated with the absence of the other). An odds ratio of 1 indicates no association; the conditions co-occur independently. Fisher's exact test (two-tailed) was used to test whether the odds ratio was significantly different from 1 (i.e., no association).

RESULTS

Nineteen of 48 (40%) primary IDCs overexpressed hsp-27, and 10 of 48 (21%) of the IDCs had TN (Table I). Of 19 IDCs that overexpressed hsp-27, 3 (16%) also had TN; in 29 IDCs with no or low hsp-27 expression, 7 (24%) also showed TN.

The odds ratio was (3/16)/(7/22) = 0.59, which suggests that if any association exists, it is a negative association. That is, IDCs overexpressing hsp-27 were more likely to be negative for TN (84%) than IDCs negative for hsp-27 (76%). However, the 95% confidence bounds for the odds ratio ranged from 0.13 to 2.64. Thus, the observations are consistent with the null hypothesis of no association (odds ratio = 1). Fisher's exact test for association returned similar results ($P = 0.72$, two-tailed); the data are consistent with the null hypothesis of no association.

DISCUSSION

Hsp-27 is among the set of polypeptides that are synthesized as a universally conserved response to stress [11,12]. Many normal and malignant tissues overexpress this protein [13], and its predictive value has been studied in a variety of cancers [14–16]. For as yet unknown reasons, the degree of hsp-27 expression has independent prognostic value in early stage breast cancer [1,2]. A better understanding of tumor-specific factors that predict high-risk disease may help define subsets of patients who will benefit most from adjuvant therapy.

Hsp-27 is overexpressed in ~67% patients with pure DCIS [5], in ~50% DCIS associated with IDC [5], and in ~25% pure IDC alone [14]. Hsp-27 expression has not been found to correlate with tumor grade [6], lymph

node status [6], ER content [3], nor HER-2/*neu* overexpression [5]. Presence of confluent necrosis in the primary breast cancer has been implicated in high-risk breast carcinoma [7], as has overexpression of hsp-27 [1,2]. We postulated that hsp-27 overexpression might correlate with the presence of TN, which might help explain why this protein is associated with reduced disease-free survival.

Alternatively, because hsp-27 is a member of a class of proteins termed molecular chaperones, which might make cells more resistant to irreversible injury and resultant necrosis [17], we considered also that there might be a negative or no association between these two prognostic factors in breast carcinomas.

CONCLUSIONS

Our evaluation of TN and hsp-27 expression in primary IDC of the breast showed no positive correlation, which we interpret as indicating a lack of association between these factors, but which supports the contention that hsp-27 expression is an independent prognostic indicator in early stage breast carcinoma.

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